Studies Of Free Living Nitrogen Fixing Bacterial Consortia For Value Added Vermicompost Production

A SYNOPSIS SUBMITTED TO

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1. **Abstract:**

Present research plan proposes to study effect of consortia of free living nitrogen fixing bacteria (NFB) isolated from various sources on the rate of manure formation. And differences in chemical nature of normal soil and vermicompost.

NFBs will be isolated from soil, organic manure and vermicompost prepared by earthworm (*sp. Eisnia foetida*). Consortium of isolated NFB will be added to raw material of vermicompost. Microbiological and chemical analysis will be done to check usefulness of consortia on agricultural crop as bio-fertilizer.

2 Introduction and literature review

Vermicompost is a non-thermophilic, bioxidative process that involves earth and associated microbes. This biological organic waste decomposition process yields the bio-fertilizer namely the vermicompost (Pathma & Sakthivel, 2012). Earthworms are capable of transforming garbage in gold: Charles Darwin described 'earthworms as unheralded soldier's of mankind' and Aristotle called them as the 'intestine of earth' as they could digest a wide variety of organic material (Darwin, Darwin, & Seward, 1903).

Earthworms excreta (vermicompost) is a nutritive organic fertilizers rich in humus, NPK, micro nutrients, beneficial soil microbes, nitrogen fixing, phosphate solubilizing bacteria, actinomycets & growth hormones gibberlins 7 cytocinine (Adhikary, 2012)

The recovery of nutrients by modification of waste like municipal solid waste, industrial solid waste, agricultural residue, animal waste, etc. is important. Recycling of organic waste through vermicompost biotechnology is an emerging trend as an 'environmentally sustainable economically valuable and socially acceptable technology' all over the world. (Pandit, Ahmad, & Maheshwari, 2012) reported that earthworms burrow's lined with earthworms cast are an excellent medium for harboring nitrogen fixing bacteria. The important basic processes weather symbiotic relation between ? micro-organism and earthworms exist in breaking down and fragmentation of organic matter progressively (Bhawalkar & Bhawalkar, 1991)

It is reported that increases in bacteria in worm and higher no. Of N-fixer's even though the total population was unaffected. (S. P. Kale, Murthy, & Raghu, 1989) (R. D. Kale, Mallesh, Kubra, & Bagyaraj, 1992).

Eisenla foetida is ubiquitous endogenic earthworm species commonly associated in manure, therefore some time known as manure worm. It require copious amount of decaying organic matter, decaying logs, compost, etc. (Roberts & Dorough, 1985).

E. Foetida can survive in normal conditions, and tolerant wide range of substances and to other physical parameter like pH, temperature, moisture (R. D. Kale et al., 1992). It is known that earthworms cannot exist on pure microbial culture but they need mixed culture of microbial species (Edwards & Bohlen, 1996).

The microbial composition changes qualitatively and quantitatively during passage through the earthworm intestine (Pedersen & Hendriksen, 1993) free living nitrogen fixing bacteria, such as Azatobacter,chroococcum& Azosprillum lipoferum,were found to have not only the ability to fix nitrogen,.but also the ability to release phytohormones similar to gibberlic acid & indole acetic acid , which could stimulate plant growth,absorption of nutrients & photosynthesis (Darzi & Rejali, 2012).

However, several anaerobic nitrogen fixer's *Clostridium butynicum*, *Clostridum beijerineki*, *Cl. parapatrificum* have been isolated from *E. foetida* (Citernesi et al., 1977).

Earlier stud mainly on, role of earthworm in breakdown of different organic waste into manure (Aalok, Soni, Tripathi, Karmegam, & others, 2009).

Organic farming by vermiculture : producing safe, nutritive and protective foods by earthworms (Sinha, Hahn, Singh, Suhane, & Anthonyreddy, 2011). Earthworm and it's excreta (vermicast) and its body wash (vermiwash) promises to second green revolution by completly replacing the destructive agro-chemicals which did more than good to both farmer's and their farm land (Sinha, Agarwal, Chauhan, Valani, & others, 2010).

Thus all these literature and many more explain the role of microbial activity in earthworm gut, cast and soil is very essential for the degradation of organic waste and release of nutrients to plants. During vermicomposting process when organic matter posses through the worm's gut, it undergoes physico-chemical and biochemical changes by combined effect on earthworms and microbial activities.

Hence the present investigation, I would to like to focus on the use of consortia (cocktail) of free living nitrogen fixing bacteria and characterization of vermicompost and normal soil under normal natural condition, and exploring of it's potential application on crops.

2. **Objectives**

The literature shows that NFB increases possibility or increasing nutrient concentration of soil. Going forward in light of literature of this topic we would like to set our objectives as follows:

- To study the free living nitrogen fixing bacteria in normal soil & organic manure.
- To study the nutrient content (NPK) in soil and organic manure.
- To study the free living NFB in vermicompost prepared by *sp*. *Eisenia foetida*.
- To study the nutrient content in vermicompost prepared by *sp. E. Foetida*.
- To study the effect on nutrient content (NPK) after pre-treatment of consortia (cocktail) of free living NFB and raw material of vermicompost.

  To study the benefit of prepared vermicompost on agricultural crop, as growth promoting agent (biofertilizer

3. **Methodology**

4.1. Isolation of free living nitrogen fixing bacteria by standard microbiological method by using specific nutrient media of normal soil, organic manual and vermicast

4.2. Chemical analysis of normal soil.

4.3. Chemical analysis of vermcompost.

4.4. Isolation of free living nitrogen fixing bacteria from vermicast.

4.5. Microbiological and chemical analysis of vermicompost resulted after pre-treatment of consortia (cocktail)

4.6. Interpretation of resulted data and analysis

4.7. To confirm the application of verrmicompost for agricultural crop by agronomical method

Importance of study

The present study mainly focuses on effect of consortia of free living nitrogen fixing bacteria on the rate of manure formation, differences in chemical nature of normal soil and vermicompost. This study would be helpful for large scale production of vermicompost from different waste byproduct in to valuable product i.e. Bio-fertilezer. This study also would be useful to clean up polluted environment in Eco-friendly manner.

4. **Significance of study**

Although lot of research groups are working on vermicompost technology and waste management. These study mostly focus on microbial flora of earthworm gut, different substrate, optimization of production process etc. but still there are some questions unanswered like what effect NFB consortia have on vermicompost process rate, nutrient content, microbial load in vermicompost as compared to normal soil, organic manure. We hope that our findings from the present study may answer these questions and will be beneficial to farmers to produce value added nutrient rich bio-fertilizer from different waste material.

5. Work plan

SR. NO.	OBJECTIVES	EXPECTED TIME
1.	Literature collection and review	0-3 month
2.	Course work preparation	3 month
3.	Isolation and microbiological study of free living (NFB) in normal soil and organic manure	6 month
4.	Chemical analysis of normal and organic manure	3 month
5.	Isolation and microbiological study of free living (NFB) in vermicompost	6 month
6.	Chemical analysis of vermicompost	3 month
7.	To study the effect of consortia in vermicomposting process, nutrient content, microbial load, etc.	6 month
8.	Effect of prepared vermicompost to agricultural crop, as a growth promoting agent.	6 month

6. References

Aalok, A., Soni, P., Tripathi, A. K., Karmegam, N., & others. (2009). Role of earthworms in breakdown of different organic wastes into manure: a review. *Dynamic Soil, Dynamic Plant*, *3*(Special Issue 2), 13–20.

Adhikary, S. (2012). Vermicompost, the story of organic gold: A review. *Agricultural Sciences*, *3*(7), 905.

Bhawalkar, V., & Bhawalkar, U. (1991). Vermiculture Biotechnology for Environmental Protection, Sustainable Agriculture Wasteland Development. *Bhawalkar Earthworm Research Institute, A/3 Kalyani, Pune-Satara Road, Pune*, *411037*.

Citernesi, U., Neglia, R., Seritti, A., Lepidi, A. A., Filippi, C., Bagnoli, G., … Galluzzi, R. (1977). Nitrogen fixation in the gastro-enteric cavity of soil animals. *Soil Biology and Biochemistry*, *9*(1), 71–72.

Darwin, C., Darwin, S. F., & Seward, A. C. (1903). *More Letters of Charles Darwin. A Record of His Work in a Series of Hitherto Unpublished Letters. Edited by Francis Darwin... and AC Seward, Etc.[With Plates, Including Portraits.]*. John Murray.

Darzi, M. T., & Rejali, F. (2012). Effects of the application of vermicompost and nitrogen fixing bacteria on quantity and quality of the essential oil in dill (Anethum graveolens). *J Med Plants Res*, *6*(21), 3793–3799.

Edwards, C. A., & Bohlen, P. J. (1996). *Biology and ecology of earthworms* (Vol. 3). Springer Science & Business Media. Retrieved from https://books.google.co.in/books?hl=en&lr=&id=ad4rDwD_GhsC&oi=fnd&pg=PR9&dq=Edwards+and+Bohlen.+1996+earthworm&ots=3agZwLWsuW&sig=Xusj9Ja5fF01ySd7cKZC1ITIIXk

Kale, R. D., Mallesh, B. C., Kubra, B., & Bagyaraj, D. J. (1992). Influence of vermicompost application on the available macronutrients and selected microbial populations in a paddy field. *Soil Biology and Biochemistry*, *24*(12), 1317–1320.

Kale, S. P., Murthy, N. B. K., & Raghu, K. (1989). Effect of carbofuran, carbaryl, and their metabolites on the growth ofRhizobium sp. andAzotobacter chroococcum. *Bulletin of Environmental Contamination and Toxicology*, *42*(5), 769–772.

Pandit, N. P., Ahmad, N., & Maheshwari, S. K. (2012). Vermicomposting Biotechnology: An Eco-Loving Approach for Recycling of Solid Organic Wastes into Valuable Biofertilizers. *Journal of Biofertilizers & Biopesticides*, *2012*. Retrieved from http://www.omicsonline.org/vermicomposting-biotechnology-an-eco-loving-approach-for-recycling-of-solid-organic-wastes-into-valuable-biofertilizers-2155-6202.1000113.php?aid=3273

Pathma, J., & Sakthivel, N. (2012). Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus*, *1*(1), 1–19.

Pedersen, J. C., & Hendriksen, N. B. (1993). Effect of passage through the intestinal tract of detritivore earthworms (Lumbricus spp.) on the number of selected Gram-negative and total bacteria. *Biology and Fertility of Soils*, *16*(3), 227–232.

Roberts, B. L., & Dorough, H. W. (1985). Hazards of chemicals to earthworms. *Environmental Toxicology and Chemistry*, *4*(3), 307–323.

Sinha, R. K., Agarwal, S., Chauhan, K., Valani, D., & others. (2010). The wonders of earthworms & its vermicompost in farm production: Charles Darwin's "friends of farmers", with potential to replace destructive chemical fertilizers. *Agricultural Sciences*, *1*(02), 76.

Sinha, R. K., Hahn, G., Singh, P. K., Suhane, R. K., & Anthonyreddy, A. (2011). Organic Farming by Vermiculture: Producing Safe, Nutritive and Protective Foods by Earthworms (Charles Darwin's Friends of Farmers). *American Journal of Experimental Agriculture*, *1*(4), 363.

Research Guide

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